

1    WHAT IS CLAIMED IS:

1                   1.       An analytical method of detecting a ligand of interest in a sample,  
2    comprising:

3                   (a)       providing a first plurality of antiligands immobilized on a solid  
4    support at positionally distinct locations thereon to provide a first array, wherein the  
5    plurality of antiligands comprises a first antiligand capable of binding specifically to a  
6    first ligand of interest;

7                   (b)       contacting the array with a sample containing or suspected of  
8    containing the first ligand, wherein the first ligand is linked through a linker to a first  
9    semiconductor nanocrystal before, during or after the contacting, under conditions in  
10   which the first ligand binds specifically to the first antiligand to form a first complex;

11                  (c)       optionally, removing unbound ligand from the array; and

12                  (d)       identifying the location of the first complex by detecting and,  
13   optionally, quantifying the presence in the first complex of the first semiconductor  
14   nanocrystal.

1                   2.       The method of claim 1, wherein the linker comprises two members  
2    of a binding pair, a first member attached to the first ligand and a second member  
3    attached to the first semiconductor nanocrystal.

1                   3.       The method of claim 1, wherein

2                   (a)       the sample contains a second ligand linked to a detectably distinct  
3    second semiconductor nanocrystal, wherein the second ligand is capable of binding  
4    specifically to a second immobilized antiligand to form a second complex; and

5                   (b)       identifying comprises determining which location or locations of  
6    the array include the first complex, the second complex or the first and second complexes  
7    by detecting and, optionally, quantifying simultaneously or sequentially the presence in  
8    the first and second complexes of the first and second semiconductor nanocrystals.

1                   4.       The method of claim 1, wherein the antiligands are nucleic acid  
2    probes and the first ligand is a target nucleic acid.

1                   5.       The method of claim 3, wherein the antiligands are nucleic acid  
2    probes and the first and second ligands are target nucleic acids.

1                   6.       The method of claim 4, wherein the first ligand is linked to the first  
2 semiconductor nanocrystal prior to the contacting step.

1                   7.       The method of claim 5, wherein the first and second ligands are  
2 linked to the first and second semiconductor nanocrystals prior to the contacting step.

1                   8.       The method of claim 6, wherein the first ligand bears a single first  
2 semiconductor nanocrystal.

1                   9.       The method of claim 7, wherein the first ligand and the second  
2 ligand bear a single first and a single second semiconductor nanocrystal, respectively.

1                   10.      The method of claim 4, wherein the linker comprises two members  
2 of a binding pair, a first member coupled to the target nucleic acid and the second  
3 member coupled to the semiconductor nanocrystal.

1                   11.      The method of claim 4, further comprising:

2                   (e)      providing a second plurality of antiligands immobilized on a solid  
3 support at positionally distinct locations thereon to provide a second array, wherein the  
4 second plurality of antiligands comprises a second antiligand capable of binding  
5 specifically to a first ligand of interest and wherein each location comprises an antiligand  
6 capable of binding to a distinct ligand of interest

7                   (f)      repeating steps (b), (c), and (d) with the second array; and

8                   (g)      comparing which nucleic acid probes from the two arrays are  
9 bound to the first ligand.

1                   12.      The method of claim 5, further comprising

2                   (e)      providing a second plurality of antiligands immobilized on a solid  
3 support at positionally distinct locations thereon to provide a second array, wherein the  
4 second plurality of antiligands comprises a second antiligand capable of binding  
5 specifically to a first ligand of interest and wherein each location comprises an antiligand  
6 capable of binding to a distinct ligand of interest

7                   (f)      repeating steps (b), (c) and (d); and

8                   (g)      comparing which nucleic acid probes from the first and second  
9 arrays are bound to the first and second ligands.

13. The method of claim 4, wherein the nucleic acid probes are allele-specific nucleic acid probes.

14. The method of claim 13, wherein

(a) the sample contains or is suspected of containing a target nucleic acid that has a first allelic site and a second allelic site;

(b) the plurality of probes includes a set of capture probes, each capture probe being capable of forming a hybridization complex with a distinct allelic form of the target nucleic acid at the first allelic site, whereby if the sample contains the target nucleic acid, then the capture probe complementary to the nucleotide at the first allelic site forms a stable hybridization complex with the target nucleic acid; and

(c) identifying comprises determining which of the capture probes are bound to the target nucleic acid.

15. The method of claim 14, further comprising contacting the target nucleic acids within hybridization complexes with a pool of nucleic acid detection probes, detection probes within the pool comprising a detectably distinct semiconductor nanocrystal and able to form a stable hybridization complex with a different allelic form of the target nucleic acid at the second allelic site, whereby a detection probe from the pool that is complementary to the nucleotide at the second allelic site forms a stable hybridization complex with the target nucleic acid; and wherein identifying comprises determining which of the detection probes is bound to the target nucleic acid.

16. The method of claim 1, wherein the plurality of antiligands are proteins.

17. The method of claim 16, wherein the ligand is a protein.

18. The method of claim 16, wherein the antiligands are antibodies.

19. The method of claim 16, wherein

(a) the sample contains a second ligand linked to a detectably distinct second semiconductor nanocrystal that is capable of binding specifically to a second immobilized antiligand to form a second complex; and

5 (b) identifying comprises determining which location or locations of  
6 the array include the first complex, the second complex or the first and second complexes  
7 by detecting and, optionally, quantifying simultaneously or sequentially the presence in  
8 the first and second complexes of the first and second semiconductor nanocrystals.

1 20. The method of claim 1, wherein the antiligand is a component of a  
2 tissue specimen.

1 21. The method of claim 20, wherein

2 (a) the sample contains a second ligand linked to a detectably distinct  
3 second semiconductor nanocrystal that is capable of binding specifically to a second  
4 immobilized antiligand to form a second complex; and

5 (b) identifying comprises determining which location or locations of  
6 the array include the first complex, the second complex or the first and second complexes  
7 by detecting and, optionally, quantifying simultaneously or sequentially the presence in  
8 the first and second complexes of the first and second semiconductor nanocrystals.

1 22. The method of claim 20, wherein the antiligands are selected from  
2 the group consisting of proteins, nucleic acid targets, oligosaccharides and combinations  
3 thereof, and the ligands are independently selected from the group consisting of  
4 antibodies, nucleic acid probes, lectins, aptamers and combinations thereof.

1 23. The method of claim 22, wherein the antiligands are distinct target  
2 nucleic acids and the ligands are nucleic acid probes.

1 24. The method of claim 22, wherein the antiligands are proteins and  
2 the ligands are proteins.

1 25. The method of claim 1, wherein the plurality of antiligands is an  
2 aptamer.

1 26. The method of claim 1, wherein the ligand is an aptamer.

1 27. An analytical method, comprising:

2 (a) providing a first plurality of nucleic acid primers having a 3' end  
3 and a 5' end and which primers are immobilized on a solid support at positionally distinct

4 locations thereon to provide a first array, wherein the plurality comprises a first primer  
5 complementary to a first target nucleic acid having an allelic site;  
6 (b) contacting the first array with a sample containing or suspected of  
7 containing the first target nucleic acid, in the presence of a first terminating nucleotide  
8 linked to a first semiconductor nanocrystal through a linker, under conditions such that  
9 the first target nucleic acid hybridizes to the first primer to form a first target-primer  
10 complex and such that if the first terminating nucleotide is complementary to the  
11 nucleotide at the allelic site the first primer is extended to incorporate the first terminating  
12 nucleotide to provide an extended primer; and  
13 (c) identifying which location or locations includes extended primer by  
14 detecting the presence therein of the first semiconductor nanocrystal.

1 28. The method of claim 27, wherein the 3' end of the primer  
2 hybridizes immediately adjacent the allelic site.

1 29. The method of claim 27, wherein the linker comprises two  
2 members of a first binding pair, the first member attached to the first terminating  
3 nucleotide and the second member attached to the first semiconductor nanocrystal.

1 30. The method of claim 27, wherein the first terminating nucleotide is  
2 a dideoxynucleotide.

1 31. The method of claim 27, wherein step (b) comprises contacting the  
2 first array with a sample containing or suspected of containing the first target nucleic  
3 acid, in the presence of the first terminating nucleotide, a second terminating nucleotide, a  
4 third terminating nucleotide and a fourth terminating nucleotide, wherein the first, second,  
5 third and fourth terminating nucleotides are linked to detectably distinct first, second,  
6 third and fourth semiconductor nanocrystals, respectively, through a linker, under  
7 conditions such that the first target nucleic acid hybridizes to the first primer to form a  
8 first target-primer complex and such that if the first, second, third, or fourth terminating  
9 nucleotide is complementary to the nucleotide at the allelic site the first primer is  
10 extended to incorporate the first, second, third or fourth terminating nucleotide to provide  
11 an extended primer, and step (d) comprises identifying which location or locations  
12 includes extended primer by detecting the presence therein of the first, second, third or  
13 fourth semiconductor nanocrystal.

- 1                   32.    An analytical method , comprising:  
2                   (a)    providing a first plurality of antiligands immobilized on a solid  
3 support at positionally distinct locations thereon to provide a first array, wherein the first  
4 plurality comprises a first antiligand that is a binding partner of a first ligand;  
5                   (b)    contacting the first array with a sample containing or suspected of  
6 containing the first ligand, whereby the first antiligand and the first ligand interact to form  
7 a first binary complex;  
8                   (c)    contacting the first binary complexes with a second antiligand  
9 wherein the second antiligand is (i) a binding partner of the first ligand and (ii) linked to a  
10 first semiconductor nanocrystal through a linker, whereby the second antiligand binds to  
11 the first ligand in the first binary complex to form a first ternary complex; and  
12                   (d)    identifying which location of the array includes the first ternary  
13 complex by detecting the presence therein of the first semiconductor nanocrystal.
- 1                   33.    The method of claim 32, wherein the first ligand is a protein.
- 1                   34.    The method of claim 32, wherein the first ligand is a nucleic acid.
- 1                   35.    The method of claim 32, wherein the first and second antiligands  
2 are first and second antibodies, respectively.
- 1                   36.    The method of claim 32, wherein the first and second antiligands  
2 are first and second nucleic acid probes, respectively.
- 1                   37.    The method of claim 32, wherein the first ligand is linked to a  
2 second semiconductor nanocrystal through a linker and step (d) comprises determining  
3 which location of the array includes the first binary complex by detecting the presence  
4 therein of the second semiconductor nanocrystal.
- 1                   38.    The method of claim 37, wherein the second semiconductor  
2 nanocrystal and the first semiconductor nanocrystal are detectably distinct.
- 1                   39.    The method of claim 38, wherein the first and second antiligands  
2 are first and second antibodies.
- 1                   40.    An analytical method, comprising:

2 (a) providing a first plurality of antiligands immobilized on a solid  
3 support at positionally distinct locations thereon to provide a first array, wherein the  
4 plurality comprises a first antiligand that is a binding partner of a first ligand;  
5 (b) contacting the first array with a sample containing or suspected of  
6 the first ligand, whereby the first ligand and the first antiligand interact to form a first  
7 complex;  
8 (c) labeling the first ligand in the first complex with a first  
9 semiconductor nanocrystal; and  
10 (d) identifying which location of the array includes the first complex  
11 by detecting the presence therein of the first semiconductor nanocrystal.

1 41. The method of claim 40, wherein:

2 (i) the first plurality of antiligands comprises a second antiligand that is a  
3 binding partner of a second ligand;

4 (ii) the sample contains or is suspected of containing the second ligand  
5 such that the second ligand and the second antiligand form a second complex;

6 (iii) step (c) comprises labeling the second ligand in the second complex  
7 with a second semiconductor nanocrystal that is detectably distinct from the first  
8 semiconductor nanocrystal; and

9 (iv) step (d) comprises determining which location or locations of the array  
10 include the first complex, the second complex or both the first and second complexes by  
11 detecting the presence therein of the first and second semiconductor nanocrystals.

1 42. The method of claim 40, wherein the first ligand comprises a first  
2 member of a first binding pair and the semiconductor nanocrystal is linked to a second  
3 member of the first binding pair by through a linker.

1 43. The method of claim 41, wherein the second ligand comprises a  
2 first member of a second binding pair and the second semiconductor nanocrystal is linked  
3 to a second member of the second binding pair.  
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